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The Joint Summer Meeting of the Anatomical Societies, held at the University of Portsmouth on 20–22 July 2010, included a symposium on 'Axon-glial Interactions in the CNS'. The following are some abstracts from the papers and presentations.

O1 Lymphatic drainage of the brain and the pathology of Alzheimer's disease

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There are no conventional lymphatics in the brain but physiological studies have revealed a substantial and immunologically significant lymphatic drainage from brain to cervical lymph nodes. CSF drains via the cribriform plate and nasal mucosa to cervical lymph nodes in rats and sheep and to a lesser extent in humans. More significant for a range of human neurological disorders is the lymphatic drainage of interstitial fluid (ISF) and solutes from brain parenchyma along capillary and artery walls. Tracers injected into grey matter drain along the basement membranes in the walls of capillaries and cerebral arteries. Lymphatic drainage of antigens from the brain by this route may play a significant role in the immune response in multiple sclerosis. Neither antigen presenting cells nor lymphocytes drain to lymph nodes by the perivascular route and this may be a factor in immunological privilege of the brain. Vessel pulsations appear to be the driving force for the lymphatic drainage along artery walls and as vessels stiffen with age, amyloid peptides deposit in the drainage pathways as cerebral amyloid angiopathy (CAA). Blockage of lymphatic drainage of ISF and solutes from the brain by CAA may result in loss of homeostasis of the neuronal environment, neuronal malfunction and cognitive decline in Alzheimer's disease. Facilitating perivascular lymphatic drainage of amyloid-β (Aβ) in the elderly as a therapeutic strategy may prevent the accumulation of soluble and insoluble Aβ in the brain in Alzheimer's disease.

O2 Impact of early life environment on the functioning of the nucleus locus coeruleus: implications for stress and affective disorders

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Different rearing conditions can permanently alter the developmental set-point of various neurochemical systems. This in turn can influence the expression of behavioural and endocrine responses to stress throughout life. Thus, early life events are thought to influence vulnerability to developing stress-induced psychiatric illnesses in adulthood. The effects of different rearing conditions on the functioning of specific brain regions in adulthood are poorly understood. Different early life environments have been modelled in rats using different durations of maternal separation during the first two postnatal weeks. One system through which maternal separation may act is the locus coeruleus (LC)–noradrenergic system that regulates emotional arousal. We demonstrate that different durations of maternal separation have distinct effects on LC physiology and dendritic morphology. Rat pups were separated from the dam for 15 min day^{-1} (HMS-15) or 180 min day^{-1} (HMS-180) from postnatal days 2–14. Others were either undisturbed (HMS-0) or were vendor-purchased controls. LC characteristics were compared at age 22–35 day using whole-cell recordings in vitro. LC neurons of HMS-180 rats were tonically activated compared to HMS-15 and control rats, with firing rates that were two-fold higher than these groups. Corticotrophin-releasing factor (CRF) application did not further activate LC neurons of HMS-180 rats but increased LC firing rate in HMS-0 and control rats. LC neurons of HMS-15 rats were resistant to excitation by CRF. Maternal separation also affected LC dendritic morphology. LC dendrites of HMS-15 rats exhibited less branching and decreased total dendritic length, an effect that could decrease the probability of contacting limbic afferents that terminate in the pericoerulear region. These results demonstrate that there are long-term functional consequences of early life events on the LC–norepinephrine system that may shape adult behaviour. Future studies are focused on understanding the changes that occur in the LC–adrenergic system at the synaptic level, including the various neurotransmitter receptors which serve to shape the level of LC neuronal activity.

O3 Tensin intracellular focal adhesion proteins: expression and roles in the brain

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The Tensin family of intracellular focal adhesion proteins, composed of Tensin1, -2, -3 and -4 (cten), are thought to act as links between the extracellular matrix and the cytoskeleton, and thereby mediate signalling for cell shape and motility. They do this via simultaneously binding to transmembrane receptors, such as integrins and receptor tyrosine kinases, as well as to the actin cytoskeleton. Such interactions are enabled by the fact that Tensins are composed of multiple domains involved in protein-protein interactions and signal transduction, such as SH2 and PTB domains. These features implicate the Tensins in cell signalling for growth, survival and cytoskeletal dynamics and consequently, dysregulation of Tensin expression has been implicated in a number of human cancers. However, nothing has been reported so far

on the expression and roles of Tensins in the brain. Recent results from immunofluorescent staining analyses in our laboratory indicate that Tensin2 and -3 show widespread expression throughout the major regions of the rat brain, and furthermore that their expression profiles are distinct from each other. This presentation shall provide a background on this family of proteins as well as show some of the results of these recent expression studies.

O4 Inward rectifier potassium channels: K^+ regulation in the optic nerve

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The inward rectifier K^+ channel Kir4.1 is almost exclusive to glial cells within the CNS, and is hypothesised to be responsible for setting the glial strongly negative resting membrane potential (RMP) during glial cell development. Here, we have examined the expression of Kir channel subtypes in both astrocytes and oligodendrocytes in a model white matter tract; the optic nerve, using immunofluorescent staining. Furthermore, we have investigated the effects of genetic modulation of Kir4.1 on whole cell conductance and Kir channel blockade on the compound action potential (CAP) and extracellular potassium ($[K^+]_o$) regulation. Mice were humanely killed in accordance with the Animals (Scientific Procedures) Act (1986) and prepared for immunohistochemistry, cell culture or electrophysiology. CAP and $[K^+]_o$ were measured whilst optic nerves were mounted in a brain slice chamber using suction electrodes and ion sensitive microelectrodes respectively. Intracellular microelectrode recordings and whole cell patch clamp analysis showed that in the absence of Kir4.1 channels, glia displayed a depolarised RMP and markedly reduced inward currents. During high frequency stimulation pharmacological blockade of Kir channels with 100 μM $BaCl_2$, increased $[K^+]_o$ to significantly higher levels than controls. Additionally, $BaCl_2$ hindered the recovery to baseline levels of both CAP amplitude and $[K_+]_o$ post high frequency stimulation. Immunostaining showed presence of other Kir channels in the optic nerve, specifically Kir 2.1 and Kir5.1, however the results demonstrate that Kir4.1 is predominantly responsible for glial cell K^+ conductance.
Supported by the MRC and IBBS.

O5 Formation of early axon tracts in the chick embryonic brain

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Initial nerve connections in the vertebrate brain form an array of longitudinal tracts, transversal tracts and commissures, from clusters of neurones. These tracts will act as a scaffold for later, follower axons that allow more complex connections to be set up in the brain. For correct development of the early axon scaffold, neurones are specified to their fate by homeobox genes such as Sax1, Emx2, Six3, Pax6 and Nkx2.1 at the correct place and time. The early axon scaffold has been identified in a number of species, and many of the tracts appear remarkably conserved between all vertebrates analysed. Although the early axon tracts have been studied in detail in many vertebrates, a direct comparison is lacking. To begin this comparative analysis a time series of the axon tracts has been done in chick using pan-neural antibody, Tuj1. The first neurones to form are labelled at the midbrain-forebrain boundary at HH11 and these will give rise to the Medial Longitudinal Fascicle (MLF). The Tract of the Post-Optic Commissure (TPOC) neurones appear next in the forebrain at HH13 and neurones of the dorsal tract of the mesencephalic nucleus of the trigeminal nerve (DTmesV) appear in the dorsal midbrain at HH14. Once the neurones have differentiated, the axons then need to project along the correct path. Axon guidance molecules are involved in the attraction and repulsion of axons. We are studying the role of Netrins in the guidance of the TPC within the early axon scaffold by ectopically expressing Netrin1 and Netrin2.
MW is supported by an Anatomical Society PhD Studentship.

O6 Signalling between placodal neurons and cranial neural crest

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In the head the peripheral sensory nervous system arises from two embryonic origins: the neural crest and neurogenic placodes. Neurogenic placodes will generate the majority of the sensory neurons, while the neural crest will generate the associated glial cells. We have shown previously that the neural crest is required for the placodal neuronal cells to migrate to form ganglia and connect correctly to the hindbrain. There is increasing evidence for a reciprocal interaction between the placodal neurons and the neural crest. Here we present data examining the role for neuregulin-1/erbB signalling in placode to neural crest signalling. In the developing cranial sensory ganglia neuregulin-1 (NRG-1) is expressed by the placodally-derived neurons, while the receptors erbB2 and erbB3 are expressed by the neural crest. To knockdown NRG1 specifically in the placodal neurons we electroporated shRNA constructs against NRG-1 into the placodal epithelium in the chick embryo. Embryos were analysed by in situ hybridisation for neural crest markers. In parallel experiments we implanted beads soaked in the ErbB inhibitor AG1478. Our results suggest that NRG-1 from the placodal neurons is required to maintain FoxD3 expression in the cranial neural crest which may reflect their commitment to a glial lineage.

POSTERS

P1 Glia and the enteric nervous system of the mouse intestine

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The enteric nervous system (ENS) is the intrinsic nervous system of the gastrointestinal (GI) tract and regulates its motor, secretory and absorptive functions. The ENS comprises an outer

myenteric plexus, which regulates motor function, and an inner submucosal plexus, which receives sensory input from the mucosa and regulates secretion, absorption and blood flow, depending on the needs of the GI segment. The ENS also contains glia, although little is known about these cells. Here, we describe the anatomical relations of enteric glia with neurons and the mucosa in the mouse intestine. Transgenic mice in which glial fibrillary acidic protein (GFAP) drives expression of green fluorescent protein (GFP) were humanely killed in accordance with the Animal Scientific Procedures Act (1986), and small intestine was fixed, sectioned and immunolabelled. Enteric astrocytes are shown to form a continuous pathway from the myenteric plexus, to the submucosal plexus, and to form a chain into the villi and to its tip. Enteric astrocytes ensheath neurons in the myenteric plexus in a manner equivalent to that observed in CNS neurons, whilst astrocytes in the villi form close associations with the mucosa. The functions of enteric astrocytes are unresolved, but our results show they form a potential communications pathway from the mucosa to the outer muscle layers. It hypothesised enteric astrocytes may have roles in neuronal activity and the immune response similar to those of CNS glia. This study shows enteric astrocytes are a major cell type in the ENS and as such are likely to be important in regulating GI function, with relevance to irritable bowel syndrome and inflammatory bowel disease.

P2 GSK3β regulation of glial differentiation in the rodent optic nerve

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Glial differentiation is a timely regulated process occurring throughout life. This process is regulated by many signal pathways important also for cell survival and cell proliferation. Glycogen Synthase Kinase 3-β (GSK-3β) is the target of numerous receptor-mediated pathways that regulate cell differentiation, however its effects on oligodendrocytes and astrocytes have not been understood. We examined the effects of GSK-3β inhibition on glial populations in the postnatal forebrain and the optic nerve: a typical CNS white matter tract. All procedures were in accordance with the Animal Scientific Procedures Act (1986). Optic nerves (ONs) aged postnatal day (P) 35 were dissected with the retina intact and maintained in organotypic culture for 3–7 days in vitro (DIV), either in normal medium or medium containing a GSK-3β inhibitor (ARA-014418, Lithium Chloride or Wnt3a). GSK-3β inhibition increased astrocytes, oligodendrocytes and oligodendrocytes precursor cells (OPCs) whereas Wnt increased OPCs but inhibited their differentiation into oligodendrocytes. GSK-3β inhibition and Wnt differentially affected GFAP+ cells, dramatically increasing their cell number and morphology. Our findings show that GSK-3β and Wnt differentially regulate oligodendrocyte and astrocyte differentiation ex vivo. This study identifies GSK-3β as a profound negative regulator of glial differentiation and we are currently examining the mechanisms of these novel GSK-3β pathways.
Supported in part by the Interreg/AdMiN project.

P3 TASK-1 channels in glial cells of the mouse CNS: implications for glial cell function

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Fundamental physiological characteristics of astrocytes are highly selective membrane permeability to K^+ and a strongly negative resting membrane potential (RMP). Tandem Pore Domain K^+ channels (K_{2P}) are accountable for the 'leak' K^+ currents that determine the RMP and membrane excitability of neurons. Therefore, we examined the role of TASK-1 in the main glial cell type in the CNS; astrocytes and oligodendrocytes. Mice were killed humanely in accordance with the UK Animals (Scientific Procedures) Act (1986), brain and optic nerves removed for immunohistochemistry, cell culture or electrophysiology. Using immunohistochemistry, we show that TASK-1 is expressed in optic nerve glia, both astrocytes and oligodendrocytes, the myelinating cells of the CNS. Patch-clamp analysis of whole cell currents was performed in glial cells cultured from mouse optic nerve explants demonstrating a component that was sensitive to the endocannabinoid TASK-1 inhibitor anandamide (10 μM) (peak currents were significantly lower at +50 mV, $P < 0.05$, t-test, $n = 7$). In addition, the current was increased at pH 8.4 and inhibited by lowering pH to 6.4 (statistically significant at +100 mV in 30 mM KCl, $P < 0.05$, t-test, $n = 5$). TASK-1 has been implicated in neuroprotection during ischemic episodes, and oligodendrocytes are particularly sensitive to hypoxia. The high level expression of TASK-1 in oligodendroglia may therefore be important in modulating oligodendroglial cell death during ischemia.
Supported by the MRC.

P4 Inward rectifying potassium channel expression and localisation in mouse glial cells

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The inward rectifier K^+ channel Kir4.1 is almost exclusive to glial cells within the CNS, and is responsible for setting the glial strongly negative resting membrane potential. The Kir4.1 channel can form heteromers with Kir5.1 and Kir2.1 channels, which modify the biophysics and rectifying power of the channels. However, little is known about functional expression of these channels in glia. Here, we have addressed this in explant cultures of optic nerve astrocytes and oligodendrocytes. Optic nerves were obtained from mice humanely killed in accordance with the Animals (Scientific Procedures) Act (1986) and prepared for explant cell culture. Immunocytochemistry demonstrates for the first time that oligodendrocytes as well as astrocytes co-express Kir4.1 and Kir5.1, consistent with their forming heteromers. In addition, but to a lesser extent than Kir4.1 and Kir5.1, astrocytes and

oligodendrocytes express Kir 2.1, a strongly rectifying channel subtype. Our results support roles for multiple Kir in glial functions, and our future studies aim to examine the mechanisms of interactions between the channel subtypes.
Supported by the MRC and IBBS.

P5 Inward rectifying potassium channel expression and localisation in human glioma cells

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Malignant gliomas present highly heterogeneous cell morphology and invasive nature and especially Glioblastoma Multiforme (GBM). Ion channels have been studied in glial cell lines and there has been research on their role in glial differentiation, growth and function. It is now recognized that the class of inward rectifying potassium channels, and especially the Kir4,1 subtype, play a significant role in the biology of mature astrocytes and oligodendrocytes, while undifferentiated, proliferating cells like malignant glioma cells do not present Kir currents. Here, we examined three major types of Kir channels, namely Kir4.1, Kir5.1 and Kir2.1. These were investigated using immunocytochemistry on three different cell lines: SNB19 (High passage GBM), UPAB (Low passage GBM) and IN699 (Cancer Stem Cells). The human GBM and CSC cell lines were obtained from the neuro-oncology lab of Portsmouth University. Our results demonstrate that high grade-high passage glioma cells (SNB19) notably express the Kir5,1 and Kir4,1 channel subtypes while showing Kir2,1 expression as well. However, the high grade-low passage glioma cells (UPAB) and cancer stem cells (IN699) show significantly lower Kir expression (9% of IN699 and 2% of UPAB cells showed Kir5.1 expression) or no expression at all (for Kir4.1 and 2.1). There is evidence that glioma cells do express Kir channels on a molecular level, but not on a functional level, as these channels seem to be mislocalized on the nucleic membrane and organelle membranes instead of the cell membrane of tumor cells (Sontheimer 2004). Our results support those findings although investigation of expression in specific organelle membranes was not conducted.
Supported by the MRC and IBBS.

P6 Localisation of inhibitory synaptic markers and GABAergic receptors in different glial populations in the CNS

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Neurons and glia are the cellular correlates of brain function. Historically, glia have been considered to provide a subordinate, supportive role to neurons in terms of overall brain function. However, a vast body of knowledge indicates the seminal role that different glial-subtypes play in normal brain function. Importantly, impairment in glial function is implicated in a number of debilitating neurological and mental illnesses. Different glia classes, which include astrocytes, microglia, oligodendrocytes and NG2 cells effect their important roles in the CNS by way of the myriad of ion channels, neurotransmitters and neurotransmitter receptors which they express. An understanding of which particular ion channels and neurotransmitter/receptors classes are expressed in functionally distinct glia in different brain regions and how this changes in different disease states is essential to elucidating the functional significant of such cell-types. We are particularly interested neuron-glia neurotransmitter signalling, especially along the inhibitory GABAergic pathways. Functional studies suggest that different glia are responsive to the effects of GABA. The distinct GABAergic receptor classes expressed on glia which are responsible for mediating such effects are poorly understood. In the current study, we provide evidence of different GABA-A receptors subunit expression in different classes of glia in different brain regions. These data will form a template for future functional analyses in health and disease.
Supported by the MRC.

P7 Immunohistochemical analysis of tensin protein expression in the mammalian brain

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The family of Tensin intracellular proteins consist of four members, namely Tensin1, Tensin2, Tensin3 and Tensin4 (cten). These are intracellular focal adhesion proteins that are thought to link the extracellular matrix, via transmembrane receptors such as integrins, to the cytoskeleton. Functionally, Tensins have been shown to play a significant role in the regulation of cell motility, and cellular growth and survival. Almost all previous investigations into the functional roles of Tensins have used somatic cells, which have uncovered a major role for Tensins in cancer cell biology. Although the expression of Tensin2 and -3 in the brain has been suggested, the expression profile of these proteins in neuronal tissue has not been determined. In the current study, we have used in-house antibodies raised against Tensins -2 and -3 to determine their regional and cellular expression profiles in the rat brain. Tensin2 showed widespread expression throughout the neuraxis, with immunoreactivity being expressed in cortical regions such as the neocortex and hippocampus as well as in subcortical regions. On the whole, immunoreactivity appeared to be punctate and appeared to be closely associated with cell membranes. Tensin2 immunoreactivity appeared to be apposed rather than co-localised with excitatory or inhibitory synaptic markers. In contrast, Tensin3 expression appeared to be concentrated in glial profiles, most notably the Bergmann glia in the cerebellum as well as in blood vessels. Taken together, the expression profiles of Tensin proteins suggest that they could play significant roles in neuronal differentiation as well regulating the synaptic environment.

P8 Distinct GABA-A receptor subunits label noradrenergic and non-noradrenergic cell-types in the locus coeruleus

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The locus coeruleus (LC) is a brain nucleus located in the dorsal rostral pons. Neurons in the LC provide the main noradrenergic innervation to almost the entire neuraxis and are instrumental in regulating the level of arousal as well as modulating adaptive behavioural responses to environmental stimuli. Furthermore, the LC is thought to modulate the cognitive limb of the stress response. Importantly, it is the level of LC neuronal activity which determines how it modulates these important brain actions. We have shown previously that different early-life environments which manifest in various behavioural phenotypes in adulthood differentially impact on the level of LC neuronal activity. Therefore, it is important to understand the synaptic factors which control the LC firing rate and in particular, the repertoire of neurotransmitter receptors effecting this modulation. In the current study, we have focused on the inhibitory modulation of LC neurons. Immunohistochemistry using confocal microscopy was used to determine the expression of different GABA-A receptor subunits in LC neurons of the adult rat brain. We are able to demonstrate that the inhibitory synaptic markers such as neuroligin-2 and gephyrin decorate various cellular profiles in the nuclear core of the LC as well as the peri-coerulear dendritic region indication a relatively homogenous distribution of inhibitory imputs across all regions of the LC. We also show that the alpha1 subunit of the GABA-A receptor is exclusively expressed in non-noradrenergic cells within the LC. These non-noradrenergic cells appear to be neurochemically diverse, expressing a range of different calcium binding proteins such as calbindin and calretinin. Furthermore, alpha1 subunit-labelled, non-noradrenergic cells appear to make contact with gephyrin-immunoreactive puncta on the principal noradrenergic cells. In contrast, the alpha3 subunit of the GABA-A receptor appears to be concentrated on the principal noradrenergic cells. In conclusion, we have shown that distinct GABA-A receptors label neurochemically diverse populations of cells within the LC. Furthermore, we hypothesise that these GABA-A alpha1 subunit-labelled non-noradrenergic neurons could belong to a pool of local circuit interneurons which might serve to modulate the activity of the principal noradrenergic neurons of the LC.

P9 Generation of GABAergic neurons in the early fetal human neocortex

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Inhibitory GABAergic interneurons make up 20–30% of the neurons in the neocortex and in humans comprise a wider variety of subtypes, including greater numbers of calretinin expressing neurons, than are seen in rodents. In addition, whereas in rodents 95% of GABAergic interneurons are born in the ganglionic eminences and migrate tangentially to their final locations in the dorsal pallium, in humans it has been estimated that about 65% of GABAergic interneurons are born locally in the cortex with a minority migrating in from subpallial locations. We have investigated expression patterns of genes associated with GABAergic interneurons and their development in human fetal brain obtained from the MRC-Wellcome Trust Human Developmental Biology Resource (www.hdbr.org), collected with written consent and ethical approval from the local Research Ethics Committee. Affymetrix® gene chip analysis of human foetal cortex samples ranging from 8 to 12 post-conceptional weeks (PCW, n = 12) found evidence of increased expression at the anterior pole of the neocortex compared to posterior pole of twenty such genes, including the transcription factors DLX2 and MASH1, the GABA synthesising enzyme GAD and calretinin. Subsequent immunohistochemical and in situ hybridisation studies on human fetal brain sections confirmed that those genes are expressed at this time in neurons and in the cortical proliferative zones, predominantly in the anterior cortex. We also saw expression of these markers in the proliferative zone and preplate from 6.5 PCW, i.e. even before the formation of cortical plate. DLX2 and MASH1 are usually associated with proliferating or recently post-mitotic cells. Observations from 10 PCW onwards show an increase in calretinin positive neurones in all cortical layers from this time. Our preliminary hypothesis is that a population of GABAergic neurons are born prior to the formation of the cortical plate in the anterior neocortex and from there these GABAergic neurons then occupy the early cortical plate. From 10 PCW a second wave of interneuron generation begins across the cortex. These hypotheses are being tested with further experiments.

P10 Increasing diameter of retinal ganglion cell axons in rat, cat and ferret and the influence of light deprivation

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The inter-relationship between an axon's diameter and the thickness of its myelin sheath is widely recognised. We have shown in various mammalian species (ferret, rat and cat), however, that the diameter of retinal ganglion cell axons is not constant along their course from the optic nerve to the optic tract – they increase in diameter. Our studies therefore addressed whether the myelin thickness also increases along the course of these axons, and whether diameter increases at a single locus or gradually along the axon's course. Further, we hypothesized that the changing diameter may reflect a mechanism for fine-tuning action potential latency to retinofugal targets, a developmental mechanism driven potentially by neural activity. We therefore asked whether the changing diameter might be influenced by early visual experience. The care and use of the animals in this study complied with both local and national regulations governing animal care. Animals were deeply anaesthetised with a lethal dose of sodium pentobarbitone, or given lethal exposure to CO_2, prior to perfusion with mixed aldehydes. Standard procedures were followed for epon-araldite embedding of segments of optic nerve and tract prior to cutting semithin and thin sections transverse to the course of axons. For

investigating the effects of light stimulus, rats were deprived of visual experience from birth for 60 days, fed under low intensity red light illumination for 10 min each day. Examination of retinofugal axons in cat shows that the relationship between diameter and myelin thickness remains constant along their course i.e. as axon diameter increases, myelin thickness increases proportionally. We also show, in rat, there is no single locus of diameter increase - the diameter increases continually along the course of the optic nerve and into the optic tract. Finally, in light-deprived rats, rather than a disruption of the increasing diameter, a relatively normal gradient of increase was observed. However, axon diameter was decreased significantly at every level measured along their course from eye to brain. Although axon diameter seems to be modulated by light exposure, the gradient of diameter increase does not appear to be influenced by environmental light levels.

P11 Permissive effects of NG2 glia on neurite outgrowth in vitro: implications for retinoic acid signalling

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Retinoic acid (RA) is a naturally occurring derivative of Vitamin A and many in vitro studies have demonstrated a role for retinoid signalling in neurite outgrowth. Cellular effects of RA are mediated via binding to the RA receptor (RAR) and retinoid X receptor, initiating transcription of target genes. Receptor subtypes and isoforms exist, and specifically RARβ2 is critical for RA mediated neurite outgrowth. The chondroitin sulphate proteoglycan (CSPG) NG2 is expressed in a population of glial cells, known as NG2-glia, that are present in both the peripheral and central nervous system. NG2-glia have been reported to support regenerating axons and interestingly, following injury they express RALDH2, the key RA synthesising enzyme. We therefore hypothesised that the RA signalling pathway is activated in NG2-glia following injury, creating a permissive environment for axonal regeneration. Optic nerves from postnatal day (P) seven mice were used to culture NG2-glia, which were then co-cultured with one of four different types of adult dissociated dorsal root ganglion (DRG) neurons: (i) preconditioned CD2019 treated, (ii) preconditioned untreated (iii) naive CD2019 treated or (iv) naive untreated. All experiments performed were in accordance with the Animals Scientific Procedures Act (1987). After 24 h co-cultures were fixed in 2% PFA and immunolabelled for NG2 to identify NG2-glia, β-III tubulin to identify neurons and RARβ. Cells were then imaged using a Zeis LSM 700 confocal microscope. The percentage of neurite bearing neurons and the length of longest neurite were calculated and high magnification analyses of neurite-NG2-glia interactions were also performed. Our results show that NG2-glia are permissive for neurite outgrowth and that the presence of NG2-glia and CD2019 treatment appears to enhance axonal outgrowth from both naive and preconditioned DRG neurons. DRG neurites are closely associated with NG2-glia and NG2-glia appear to influence the direction of neurite outgrowth. In addition both NG2-glia and dissociated DRG express RARβ. These results suggest that NG2-glia may enhance axonal outgrowth in vitro via the RA signalling pathway although further studies are required to elucidate the molecular mechanisms behind this effect.

P12 Metastasis studies of the blood-brain barrier using in vitro models

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Introduction: Around 25% of cancers will spread to the brain by passing through the blood-brain barrier (B-BB) and thereby worsen prognosis. In addition the B-BB has proven to be a serious obstacle to therapeutic delivery. In vitro models of the B-BB generally utilise mixed species, non-human cells and can be difficult to extrapolate to the human in vivo situation. The Electric cell-substrate impedance sensing system (ECIS™) and dynamic in-vitro-blood brain barrier (DIV-BBB) models are novel techniques which can be used to monitor the B-BB components more effectively than traditional Transwell® models.

Methods: The B-BB model was comprised of human astrocytes (CC-2565 and SC-1810) with human cerebral microvascular endothelial cells (hCMEC/D3) under human serum supplementation in a Transwell® system. Cells were characterised with appropriate immuno-markers using flow cytometry and immunocytochemistry. ECIS™ was investigated to monitor the hCMEC/D3 grown on a range of extracellular matrices (ECMs), with conditioned media (CM), and cancer cell invasion. DIV-BBB was used to monitor the difference media flow had on hCMEC/D3.

Results: Growth on Transwell® systems and trans-endothelial electrical resistance (TEER) measurements were established. ECIS™ demonstrated the potential of hCMEC/D3 to form a tight barrier. Astrocytes were shown to have no positive effect when added either as cells under the hCMEC/D3 monolayer, or as astrocyte-derived ECM but did increase TEER values when added as conditioned medium. Malignant cells placed upon hCMEC/D3 monolayers were shown to have metastatic potential through the hCMEC/D3 monolayers and recordings were taken on ECIS™. The DIV-BBB model incorporating hCMEC/D3 cells recorded high TEER values.

Conclusion: An all-human in vitro B-BB model has been developed using a Transwell® co-culture system and monitored with the use of ECIS™. The DIV-BBB model has shown the potential to become a novel B-BB model.

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P13 Immunotoxin Ablation of NG2 and GD3A: potential novel approach to glioma treatment

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Introduction: The molecule neuron-glia 2 (NG2) is a transmembrane chondroitin sulphate proteoglycan that is present on a distinct population of glial cells in the central nervous system

(CNS). NG2 is often re-expressed by brain tumours and has been found to have increased expression levels in high grade glioma-derived cell lines. Our studies suggest differential functions of NG2 and GD3 on the same glioma cell populations, with NG2 expression confering increased proliferation, while a 'switch' to GD3A facilitates tumour invasion.

Methods: Glioma derived cell lines UPAB, UPMC and UPPP were seeded at a density of 1×104 in 96 well plates and were treated firstly with NG2 antibody immunotoxin conjugate (Mab-Zap) and incubated for 72 h, then sequentially treated with GD3A antibody immunotoxin conjugate and incubated for a further 72 h. The primary antibody concentration was maintained at 1 : 1000 and a range of secondary immuntoxins concentrations were used (0–5 μg mL^{-1}). After the incubation periods cells were exposed to the MTS cell proliferation assay. This secondary immunotoxin approach comprised of primary antibodies directed against NG2 and GD3A being conjugated to a secondary antibody with the immunotoxin Saporin attached.

Results: The highest concentration of Mab-Zap (5 μg mL^{-1}) combining both NG2 and GD3A primary antibodies resulted in approximately 94% of cell death in all three cell lines UPAB, UPMC and UPPP.

Conclusions: Proof of principle has been demonstrated that NG2 & GD3A positive gliomas may be selectively and effectively ablated using a secondary immunotoxin approach. This involvement of NG2 & GD3A in multiple aspects of tumour biology makes these molecules attractive candidates for future therapies against malignant glioma. NG2 may provide a suitable target for cytotoxic therapy, particularly when harnessed with approaches which aim to target the shared glioma cell antigen GD3A.

P14 Targeting GD3/GD3A in novel therapy development for glioma

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Introduction: The ganglioside GD3 is upregulated in neoplastic cells and plays a pivotal role in the regulation of tumour growth and invasion. Whilst in non-neoplastic cells, the build up of GD3 induces mitochondrially-mediated apoptosis, this does not occur in tumour cells due to the acetylation of the terminal sialic acid to form GD3A. Haemaglutinin esterase (HE) from Influenza C virus has been shown to deacetylate GD3A and restore pro-apoptotic GD3. We are investigating the efficacy of exogenous addition of recombinant HE and transfection and transduction with the HE at deacetylating GD3A in gliomas and inducing mitochondrially-mediated apoptosis.

Methods: Recombinant HE and HE were supplied by R. Vlasak, cells used in all assays are glioblastoma multiforme biopsy-derived early passage cells and normal human astrocytes are used as a control. GD3/GD3A expression was determined by flow cytometry and immunocytochemistry, Cell viability was determined using the MTS and Annexin V assays western blotting for cytochrome c. The effect on invasion was assessed using the modified Transwell Boyden Chamber™ assay.

Results: Exogenous addition of recombinant HE causes a decrease in expression of GD3A and an increase in GD3 expression, this occurs with a simultaneous increase in cytotoxicity and apoptosis and a decrease in invasion. We have generated pcDNA3.1-HE and pBacPAK-CMV-HE for transfection/transduction studies. Our previous studies have shown the baculovirus to be effective at transducing brain tumour cells.

Conclusions: HE deacetylates GD3A in glioma to a critical threshold and restores apoptotic ability. GD3A and GD3 are onco-fetal antigens and are not expressed in astrocytes thus HE has no effect on these cells. Deacetylation of GD3A may be a potential therapeutic strategy for glioma.

P15 Influence of hypoxia on cellular biology in CD133-expressing glioma cells

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Introduction: CD133, a 120 kDa transmembrane spanning glycoprotein, has been identified as a marker for a subpopulation of neural cancer stem cells; an assumption which has been hotly debated in the neuro-oncology research community. Solid tumours characteristically contain poorly vascularised areas associated with severe hypoxia, usually related to poor patient prognosis. The functional role of CD133, as well as the influence of oxygen tension on various biological parameters including adhesion, migration, invasion and proliferation has, therefore, been assessed in this study.

Methods: CD133-positive and negative cell fractions were isolated from a paediatric GBM by magnetic bead immuno-cell segregation (AutoMACS™) and fluorescence activated cells sorting (FACS™). The cells were cultured with stem cell defined growth medium supplemented with the appropriate growth factor concentrations and maintained under hypoxic conditions at 3% oxygen and 5% carbon dioxide. The positive and negative cell fractions were characterised using flow cytometry and immunocytochemistry with the CD133/1 (AC133) monoclonal antibody. Migration and invasion was studied using transwell™ Boyden chambers plus the various ECM substrates. Proliferation analysis was conducted using bromodeoxyuridine (BrdU), proliferatiing cell nuclear antigen (PCNA) and the monoclonal antibody Ki-67. A differentiation adhesion assay with various extracellular matrices, including vitronectin and fibronectin, was also used. Human neural stem cell differentiation arrays were used to assess the progenitors of the positive and negative cells fractions.

Results: A reduction in the levels of oxygen was seen to dramatically affect proliferation rates, cell migration and invasion rates, as well as adhesion. The positive fractions, although displaying an increased proliferation index in comparison to the negative fraction, showed a reduced invasion and migration rate as opposed to the negative CD133 cells.

Conclusions: The in vitro hypoxic microenvironment dictates the behaviour of cultured neoplastic glia as well as dramatically influencing the CD133 phenotype. Distinct biological differences are apparent between the CD133-positive and CD133-negative cell populations. Whether or not CD133 accurately defines a cancer stem cell within glioma remains to be determined but CD133

expression does relate to oxygen environment and biological properties including proliferation, adhesion and invasion.

P16 A functional study of CD44 and CD155 in glioma invasion

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Introduction: Increased expression levels of CD44 (Hyaluronic acid/lymphocyte homing receptor) and CD155 (Poliovirus receptor) have been reported on human glioma cells. While CD44 has long been associated with tumour invasion, CD155 has more recently attracted interests in a similar context.

Methods: Immunocytochemical and flow cytometric expression of CD44 and CD155 was established on established cell lines and early passage cultures of biopsy-derived glioma. Total Internal Reflected Fluorescence (TIRF) microscopy enabled high signal/low noise imaging of double labelled cells. The effects of monoclonal antibody blocking and siRNA silencing on CD44 and CD155, both individually and together, were assessed using Transwell assays and live cell imaging for invasion, motility and velocity of cell movement. BrdU cell proliferation assays were used to assess the proliferative in siRNA knockdown cells. Interaction and localisation of CD44 and CD155 with F-actin and integrins (α1, $\alpha v\beta$1 and $\alpha v\beta$3) were shown by confocal microscopy.

Results: CD44 was expressed evenly across the cell surface while CD155 sometimes accumulated in 'zones' over the cell surface and at the leading edge of invadopodia. TIRF microscopy revealed close proximity between the two epitopes, albeit at distinct sites on the cell surface. CD44 blocking and silencing resulted in a higher level of inhibition of invasion than for CD155; such interference with combined CD44/CD155 resulted in 100% inhibition of invasion within the time frame of the studies. Live cell imaging showed a reduced motility and velocity in cell movement of knockdown cells. Higher proliferative rates were seen in siRNA /CD44 and siRNA/ CD155 cells. Confocal microscopy showed distinct overlapping of CD155 and integrins on filopodia.

Conclusions: Monoclonal Antibody blocking and siRNA knockdown of CD44 and CD155, both singularly and in concert, reduced invasion and increased proliferation in glioma cells. Joint CD44/CD155 approaches may merit further study in targeting infiltrating glioma cells in therapeutic protocols.

P17 Glycogen synthase kinase 3 expression in murine tissues

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Activity of glycogen synthase kinase (GSK) 3 beta causes inhibitory phosphorylation of beta catenin in the Wnt signalling pathway. The kinase acts by adding phosphate molecules to serine and threonine amino acids on certain cellular substrates. Wnt signalling helps to regulate beta catenin activation and translocation to the nucleus that initiates downstream transcriptional processes leading to for example proliferation. Given the proliferative activity that occurs in gut epithelium, the immunohistochemical expression of GSK3 beta was examined in the murine digestive tract and other tissues. GSK3 beta expression was present in the glandular epithelium of the stomach, small intestinal crypts and villi but absent in the liver, spleen, kidneys and testis. The histological localisation of GSK3 beta in the stomach and intestine suggests that this kinase is not widely distributed in the epithelial cells in the gut.

P18 A quantitative investigation of transcriptional activity in syncytiotrophoblast nuclei during human gestation

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The syncytiotrophoblast (STB) is a terminally differentiated, multi-nucleated syncytium. No mitotic bodies are observed in the STB, which is sustained by continuous fusion of cytotrophoblast cells (CTB). As a result, STB nuclei are of different ages and display varying degrees of heterochromatin. Until recently, it was thought that STB nuclei were transcriptionally inactive, with all mRNAs required by the syncytium being incorporated upon fusion of CTB. However, research now shows the presence of the active form of RNA polymerase II (RNAP) in some STB nuclei (Ellery, Placenta, 30, 2009). The aim of this study was to quantify the proportion of transcriptionally active nuclei at different gestational ages. Paraffin-embedded placentas (n = 22), ranging from 13 to 39 weeks, used for the estimation of STB nuclear number (Simpson, Placenta, 13, 1992) were studied. For each placenta three blocks were selected at random, and adjacent 5 μm sections cut. The proportion of RNAP-positive STB nuclei was quantified by the Disector method. Numerical densities of volumes of trophoblast sampled were calculated. Co-localisation of Proliferating Cell Nuclear Antigen and RNAP was used to investigate the relationship between recently fused nuclei and RNAP positivity. There was no correlation between gestational age and numerical density of RNAP positive nuclei ($r^2 = 0.0765$, $P = 0.2248$). The numerical density remained constant throughout gestation. These findings have shown that transcription takes place in a proportion of STB nuclei throughout gestation. Since the number of STB nuclei increases exponentially (0.62×1010 nuclei at 13–15 weeks to 5.81×1010 at 37–39 weeks) and the numerical density of RNAP positivity remains constant, we conclude the number of transcriptionally active nuclei also increases exponentially. Further research is needed to determine the mechanisms controlling the maintenance of heterochromatin in STB nuclei including investigating chromatin modifications and heterochromatin-binding proteins.

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P19 Leptin and leptin receptors in the rat and human carotid body

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Leptin is known to play a role in modulation of metabolism and control of breathing mainly acting on central nervous structures. Notwithstanding, additional actions on peripheral arterial chemoreceptors have also been suggested in the literature. Thus, in the present study we investigated, through immunohistochemistry, the expression of leptin and leptin receptors in the carotid bodies of humans and Sprague-Dawley rats. Leptin expression and relative expression of the leptin receptor isoforms Ob-Ra, Ob-Rb, Ob-Rc, Ob-Re, Ob-Rf were also investigated in rats by real-time Polymerase Chain Reaction (real-time PCR). The study was performed in accordance with the Italian Public Health Office regulations and under appropriate ethical committee approval. No leptin and leptin receptor immunoreactivities were visible in the type II cells of both series. Conversely, anti-leptin immunoreactivity was found in type I cells of both humans and rats, respectively. Diffuse positive staining for leptin receptors was also observed in human and rat type I cells, both with an antibody specific for isoform Ob-Rb and with an antibody recognizing all receptor isoforms. Real time-PCR showed the expression of leptin and Ob-Ra, Ob-Rb, Ob-Rc and Ob-Rf isoforms mRNA in the carotid body, levels of expression being as follows: Ob-Rf > Ob-Rc >> Ob-Ra >> Ob-Rb. Ob-Re mRNA was not detected. The above findings suggest a role of circulating or locally producted leptin in the regulation of chemoreceptor discharge through direct action on type I cells and/or indirect vasoactive effects on carotid body microvessels. A possible modulatory action on carotid body glucosensing function may also be hypothesized.

P20 Proposition of a new statistical method for facial reconstruction in forensic medicine

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In recent years, the development of medical imaging has had a major impact on facial reconstruction. New strategies have been proposed to reconstitute the morphology of a face from the observation of a skull. Usually, these techniques are based either on few landmark measurements or on the use of templates associated to the face and the skull. In our work, we choose a local and individual approach based on the used of dense meshes associated to a large collection of landmarks directly extracted from CT-scans. Our method allows to reconstruct local features on the skull like the nose with a good accuracy. We first built a database with 47 CT-Scan using whole head performed on 47 volunteers European women aged from 20 to 40 years. Our image processing includes 1/the segmentation of both skull and external skin surface for each slice; 2/the construction of two 3D surfaces by meshing curves on successive slices. Then 39 landmarks are manually located on each skull mesh. Our image processing step allows to compute geodesics on the meshed surface and extract anatomically identified feature from the bone surface (bone patch). Using registration techniques it is possible to construct a distance between individual features on the skull (bone patch) and to compute average of the corresponding skin features. We have derived two approaches to compute such average of skin features : one is based on the extraction of skin thickness, the second is based on the extraction of the external skin surface.